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Towards a Modular, Robust, and Portable Sensing Platform for Biological and Point of Care Diagnostics

by Amethist S. Finch*, Justin R. Bickford, Marvin A. Conn, Matthew B. Coppock, Deborah A. Sarkes, and Dimitra N. Stratis-Cullum

ARL-RP-0449 **July 2013**

A reprint from the *Proceedings of SPIE* vol. 8719 87190P-1.

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REPORT DOCUMENTATION PAGE	Form Approved OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED (From - To)
July 2013		

4. TITLE AND SUBTITLE
Towards a Modular, Robust, and Portable Sensing Platform for Biological and Point of Care Diagnostics

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Amethist S. Finch*, Justin R. Bickford, Marvin A. Conn, Matthew B. Coppock, Deborah A. Sarkes, and Dimitra N. Stratis-Cullum

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Army Research Laboratory
ATTN: RDRL-SEE-B
2800 Powder Mill Road
Adelphi, MD 20783-1197

8. PERFORMING ORGANIZATION REPORT NUMBER
ARL-RP-0449

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
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15. SUBJECT TERMS
Point of Care, Molecular Recognition, Hand-Held Device, Smart Phone, Ubiquitous Sensing

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Amethist S. Finch
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UU	12	19b. TELEPHONE NUMBER (*Include area code*) (301) 394-0326

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

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ABSTRACT

The ability to conveniently and immediately test and diagnose in a diverse and rapidly changing environment is critical for field diagnostics. Smart biomedical sensors employ many different diagnostic/therapeutic methodologies; however, an ideal system would include the ability for results to be shared instantaneously with all members of the team through a software interface. We discuss our efforts towards the development and use of a robust, mobile platform (Android-based smart phone) that incorporates stable molecular recognition elements in sensor development. The inexpensive, compact, robust, archival, and portable design is ideal for rapid field diagnostics.

Keywords: Point of Care, Molecular Recognition, Hand-Held Device, Smart Phone, Ubiquitous Sensing

1. INTRODUCTION

Point of care (POC) testing has the potential to provide immediate diagnostic results to mobile personnel (physicians, patients, medics, etc), allowing the administration of treatments as quickly as possible. Effective POC tests require portable devices capable of measuring specific targets like proteins, nucleic acids, and drugs at potentially very low concentrations in a variety of complex media such as saliva, blood, urine, and other bodily fluids. These handheld gadgets have the potential to lower the cost of diagnosis and save immense amounts of time by removing the need to collect, preserve, and ship samples to a secondary facility. An ideal POC device should be easy to use, be adept at monitoring a variety of analytes, provide consistent performance regardless of atmospheric conditions, and have the capability of uploading results to a dedicated network.

There have been a number of recent reviews discussing the current POC technologies that incorporate many characteristics of the "ideal" device.[1-6] The most commercially successful example of a POC device is the electrochemically based glucose monitor, making up about 85% of the biosensor market.[7] The success of this device can be attributed to its ease of use, accuracy, and rapid analysis, which can take as little as 5 s on blood volumes as low as 300 nL.[4] Additionally, lateral-flow assays, such as pregnancy tests, disease and drug abuse screens, and blood protein markers, exhibit widespread use. Recent parallel advances in on-chip diagnostics, microfludics, and smartphone platform technologies have enabled state-of-the-art examples that are being studied and improved upon.[8]

A number of key factors are driving research momentum towards the utilization of smartphone technology, including the necessity to remove the human factor in analysis of test results, especially from more subjective tests such as colorimetric assays. Figure 1 illustrates the components that are necessary for fully integrated POC devices, and the linchpin in the broad base adoption of this technology is the smartphone. Smartphones are well-poised to revolutionize the POC field due to the additional onboard sensors such as advanced optics, GPS, an accelerometer, an electromagnet, a gyro, a proximity sensor, and a barometer. The added onboard functionality of modern smartphone devices further empowers fielded personnel for the potential of rapid onsite diagnostics. Another

Smart Biomedical and Physiological Sensor Technology X, edited by Brian M. Cullum, Eric S. McLamore,
Proc. of SPIE Vol. 8719, 87190P · © 2013 SPIE · CCC code: 1605-7422/13/$18 · doi: 10.1117/12.2014766

advantage to a device coupled to a smartphone is the ability to upload comprehensive data to a dedicated network, not only to archive patient results, but also allow easy access to study trends and locations of medical emergencies by use of the GPS integrated in the phone. We are currently working on the production of a handheld, field-portable device with archival capabilities for ubiquitous sensing applications, including POC diagnostics. Specifically, the focus of this paper is to highlight recent work in synthetic capture reagents that will further enable embedding the technology into a smartphone platform for POC diagnostic applications.

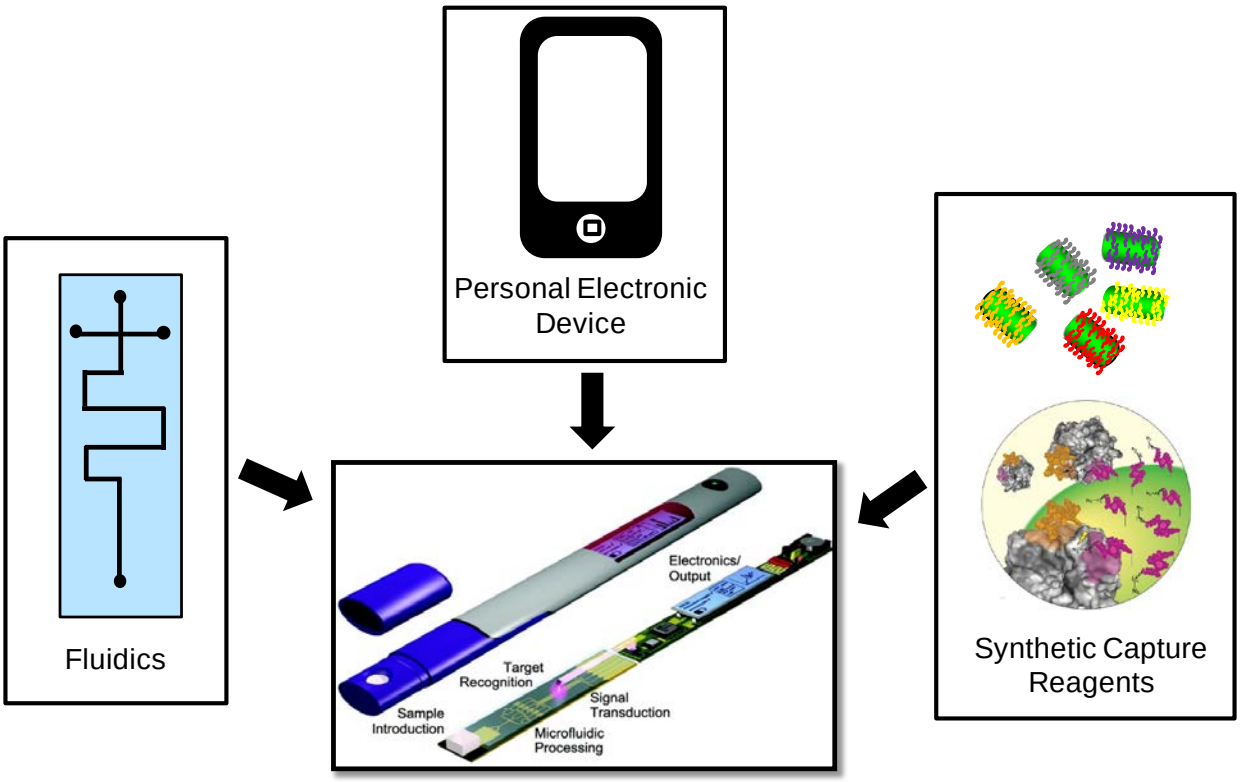

Figure 1: Diagram outlining examples of the key components (fluidics, personal electronic device, and synthetic capture reagents) to a fully integrated POC device. The idealized POC device figure was reproduced with permission from John Wiley and Sons, Inc.[1]

2. EXPERIMENTAL

2.1 Materials

All chemicals and supplies were purchased from Sigma-Aldrich, Fisher Scientific, Bio-Rad, or Invitrogen, and were the highest grade and purity available. All aqueous solutions were prepared with purified nanopure water, and solutions were sterile-filtered or autoclaved prior to use. Peptide sequences were chosen for their affinity to protective antigen (PA) of *Bacillus anthracis* as described in the literature.[9] Bi-ligand was provided by the laboratory of Professor James Heath (Caltech, Pasadena, CA).

2.2 Methods

Sorting Procedures and Sample Preparation – Figure 2 illustrates a general schematic of the bacterial display library and the generic steps in the biopanning process (Cytomx Therapeutics; San Francisco, CA: eCPX library) which contains approximately 3×10^{10} members. The biopanning process for selection of isolates that display PA binding peptides was conducted as previously described.[9-11]

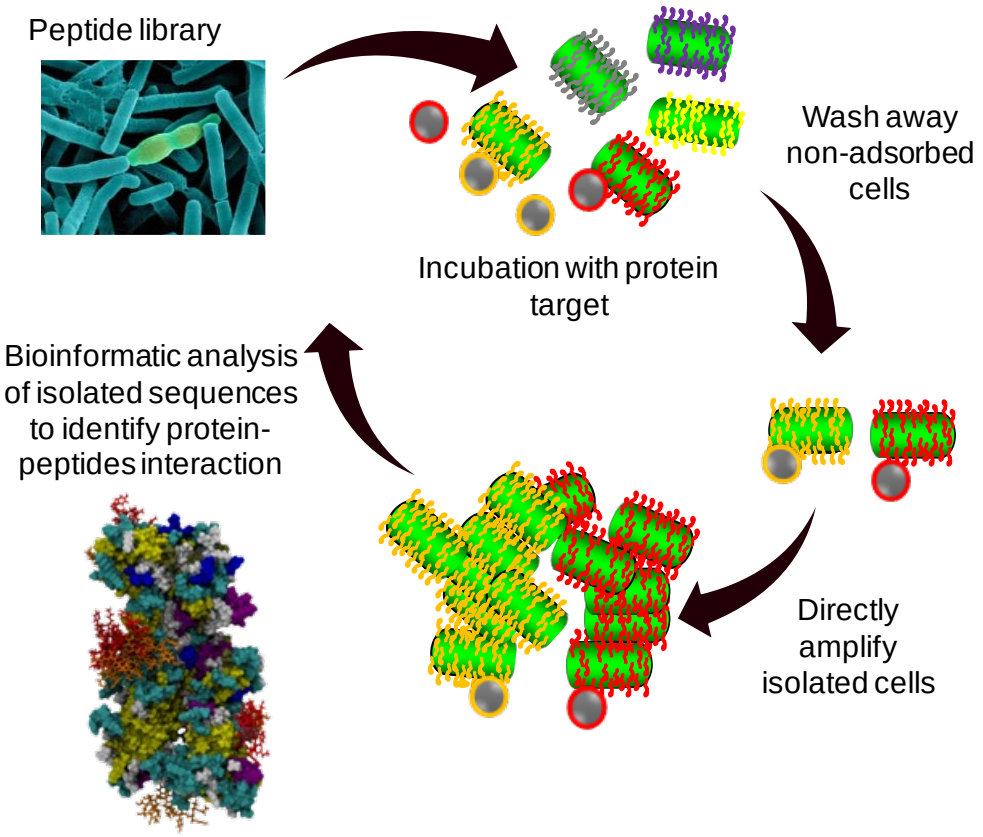

Figure 2: Conceptual depiction of the biopanning process with bacterial display technology. Randomized 15-mer peptide is displayed on the outer membrane of *E.coli* yielding approximately a 10^{10} member library. Through a series of stringency washes and amplification steps the library is evolved to bind to a target of interest.

Anchor and Bi-ligand development – Figure 3 illustrates the general screening process for preparation of a multi-ligand protein-capture agent as described previously in the literature.[12]

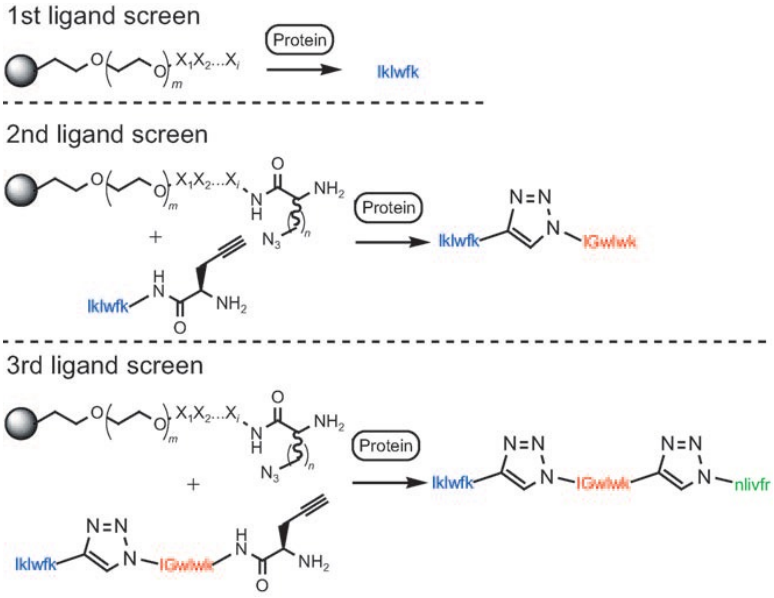

Figure 3: Schematic representation illustrating the steps necessary for preparation of a multi-ligand protein-capture agent. Figure reproduced with permission from John Wiley and Sons, Inc. [12]

Circular Dicroism (CD) Spectroscopy – CD measurements were performed on a Jasco J-815 CD spectrometer. Eighty nanometers of peptide in 10 mM potassium phosphate buffer pH 8.0 was heated from 20 ^{o}C to 70 ^{o}C, with a temperature gradient of 1^{o}C/min and a 10 sec delay time. The sample was heated and cooled a total of four times, and a spectrum was collected every 10 ^{o}C only during heating. Each spectrum was accumulated three times, with continuous scanning at a speed of 20 nm/min between 260 nm and 190 nm. The data were smoothed using the negative exponential algorithm in SigmaPlot 12.

3. RESULTS AND DISCUSSION

We will discuss the latest results in the three key areas (personal electronic devices, advances in synthetic recognition elements, and fluidics and materials integration) necessary for development of a comprehensive POC diagnostic toolkit. Initial biological target focus is on protective antigen (PA) of *Bacillus anthracis* using synthetic peptide antibody replacements coupled with the development and optimization of the sensor platform. Future candidate selection will be based on operational need.

3.1 Personal Electronic Device

A brief investigation of commercial smartphones was conducted with the goal of indentifying and recommending an Android-based smartphone for onboard image capture and image processing algorithms as the base for the ubiquitous sensing platform. There is momentum in the military research community to make use of Android-based phones primarily because of their open source availability. Android's source code and the Army's NETT Warrior program are accelerating incorporation timelines.[13] The NSA is doing research and has modified the Android platform to provide highly secure cellular communications for military applications.[14] In contrast, the iPhone is a more guarded platform that does not allow developer access to the operating system source code for modification.[15] As a result, use of Android-based phones for R&D is a very reasonable choice and in reviewing available choices, the *Samsung Galaxy S3* (SGS3) was down selected. The SGS3 has the greatest potential for memory storage and uses the ARM quad core technology for parallel processing, which is supported by Android. In addition, the SGS3 has many additional sensors such as a GPS, an accelerometer, an electromagnet, a gyro, a proximity sensor, and a barometer. These onboard sensors may provide for future opportunities to augment the functionality of the POC device. Table 1 highlights these key desirable design features.

Table 1: Desired features for smartphone selection

Desired Features	Enabling POC Diagnostics
Standardized	Universal interface capable of operation across platforms
Open & Programmable	Necessary for graphical user interface and software application to be rapidly modifiable
SWAP-C	For widespread use, platforms must meet economic demands, including size, weight, power, and cost
Standard Capabilities	Integrated archival data storage (GPS, time, date, readout, images, etc.) available for after-action processing
Networked	Data transmitted and recorded for further analysis and processing
Easy to Use	Operation and readout on platform must be simple for broad acceptance
Multiplexed Analysis	Extendable to include biothreat sensing, POC diagnostics, small molecule (cocaine, TNT, etc.), and nuclear materials

3.2 Advances in Synthetic Capture Reagents

In previous work, we illustrated the use of an eCPX-based bacterial display library for the rapid screening and selection of peptide synthetic recognition elements (SRE) to biological targets of interest.[9, 16, 17] The eCPX library contains approximately 3×10^{10} members, with each bacterial cell displaying a discreet 15-mer peptide that

is on the outer membrane surface of *E. coli*. Through a series a series of stringency washes and amplification steps, targets of interest are screened for binding affinity. While in direct comparison with antibody technology, though the biopanned15-mer peptide SREs tend to vary in specificity and affinity, they hold promise in stability and manufacturability. These two elements—stability and manufacturability—are critical for POC applications, not only to address field environmental conditions, but also for combating newly emerging threats. Table 2 highlights a number of key features that are desirable for reagents for optimal integration into POC diagnostic tools.

Table 2: Key features of reagents for integration into POC diagnostics tools.

Desired Feature	Necessary for POC Diagnostics
Robust	Temperature, pH, enzyme degradation, and a long shelf life
Thermoplastic	Maintains full function under extreme temperature conditions
Rapid Discovery	Rapid development without extensive knowledge of the target analyte, critical to adapt technology to new and emerging threats
Manufacturing Scale Production	Cost-effective on demand production necessary for ubiquitous scale
Low Cost	Critical for universal implementation
Adaptable	Readily incorporate into variety of platforms; drop-in replacement technology
High Affinity	Equivalent (or better) than antibody gold standard to meet sensing requirements
High Specificity	Critical for practical application in complex environments

To further address the stability and manufacturability, the next generation of SRE will be directly coupled with protein catalyzed capture (PCC) agents. PCC agents are evolved via an in-situ catalyzed click chemistry reaction, allowing synthetic peptides to be joined, creating new molecules with antibody-like properties.[12, 18] This direct coupling of SRE and PCC agent technologies will not only address stability and manufacturability but also bridge the selectivity and affinity gap. Since the PCC agent screening process involves assembly using an *in situ* click process that is catalyzed by the protein target itself, affinity and selectivity is built in from the start. Additionally, this technology allows for screening of additional characteristics including protease resistance (can be constructed from natural, non-natural amino acids) and epitope targeting.

In order to directly observe the ability of SRE's and PCC Agents, an SRE-developed 15-mer peptide and PCC Agent bi-ligand were both tested for their thermoplasticity. Figure 4 illustrates that after several heating and cooling cycles, both the stand-alone 15-mer peptide (Figure 4A) and the PCC Agent bi-ligand (Figure 4B) were able to return to their initial structure. This data is very promising and suggests that these materials may retain their function even after repeated thermal cycling or storage under elevated conditions. Further testing is ongoing and focuses on coupling activity assays with increasing temperature and cycle numbers.

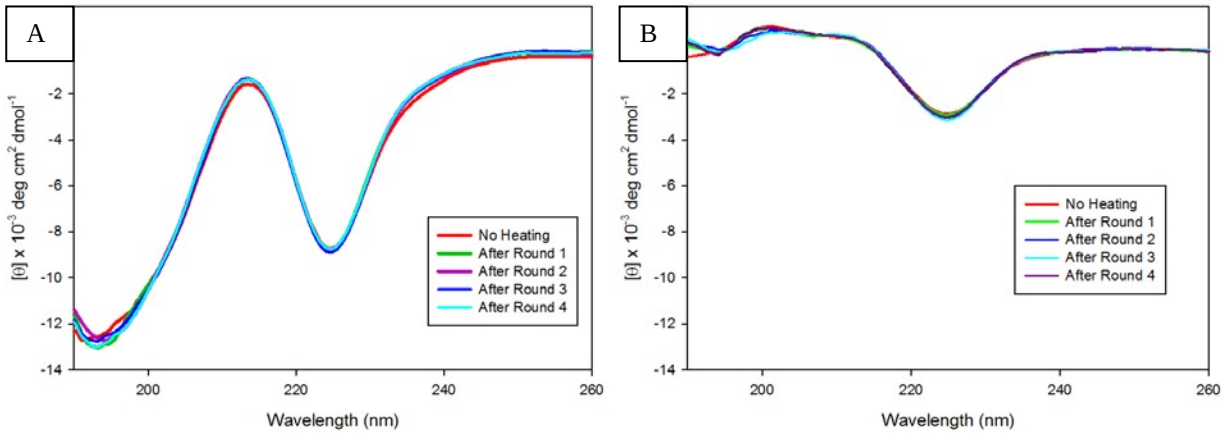

Figure 4: Thermoplasticity studies using CD spectroscopy (A) PA SRE 15-mer peptide (B) PCC Agent bi-ligand.

3.3 Fluidics and Materials Integration.

A number of different technologies have been suggested and discussed for engineering the idealized POC device. These competing technologies range from fluorescence conjugated optofluidics-based detection schemes[8, 19] to direct incorporation into hand-held assays by gold colloid conjugation.[20] Figure 6 illustrates a hand-held assay schematic that could potentially be modified for use with SRE replacing antibody technology. Future work includes incorporation of stable and manufacturable synthetic recognition element (i.e., PCC Agent) with a transduction element (i.e., quantum dot) for onboard detection via smartphone.

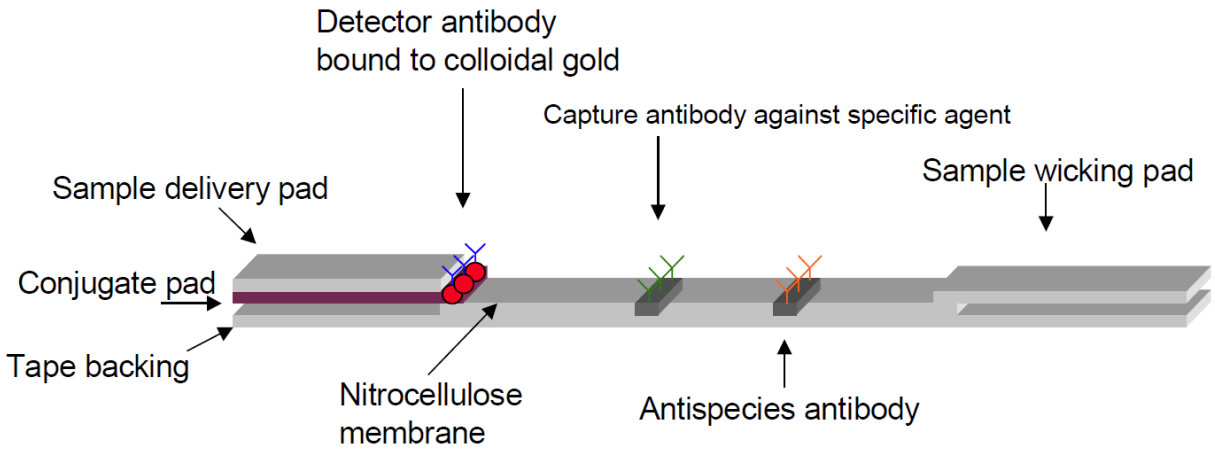

Figure 5: Generic diagram illustrating a hand-held assay test strip.

4. CONCLUSION

To conclude, we highlight examples of the ongoing fundamental work in our laboratory towards the development of a diagnostic toolkit that will enable rapid and ubiquitous POC diagnostics. Through the combination of smartphone technology, synthetic capture reagents, and fluidics and materials integration, this research should ultimately allow for advancement and evolution of the next generation of mobile handheld diagnostics tools. Although the rapid development in the area of fully integrated POC devices is in its infancy, the promise of the future potential is already greatly evident in the literature. If innovations persist at the current pace, over the next decade these exciting trends will continue and further evolve the ability to conveniently and immediately test and diagnose in diverse and rapidly changing environments.

5. ACKNOWLEDGEMENTS

The authors acknowledge the support of the U.S. Army Research Laboratory, Sensors and Electron Devices Directorate (ARL-SEDD) and the ARL-SEDD SEED program for funding. Research is supported in part by appointments (M.C.) to the *U.S. Army Research Laboratory Postdoctoral Fellowship Program* administered by the *Oak Ridge Associated Universities* through a contract with the *U.S. Army Research Laboratory*. D.S. acknowledges Goldbelt Raven, LLC for administrative support.

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1 (PDF)	ADMNSTR DEFNS TECHL INFO CTR ATTN DTIC OCP (ELECTRONIC COPY) 8725 JOHN J KINGMAN RD STE 0944 FT BELVOIR VA 22060-6218
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